

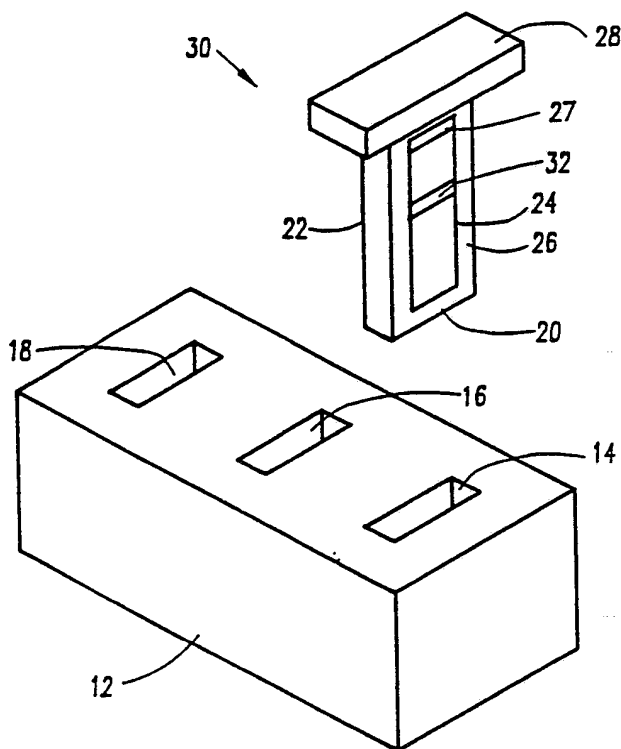


## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/NL92/00176 <b>(22) International Filing Date:</b> 5 October 1992 (05.10.92)  <b>(30) Priority data:</b> 99647                      4 October 1991 (04.10.91)      IL 102486                    13 July 1992 (13.07.92)        IL  <b>(71) Applicant (for all designated States except US):</b> ORGENICS INTERNATIONAL HOLDINGS B.V. [NL/NL]; c/o TMF Management B.V., Emmaplein 5, NL-1075 AW Amsterdam (NL).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> REINHARTZ, Avra- ham [IL/IL]; 1 Schachar Street, Rehovot (IL). ALAJEM, Sarah [IL/IL]; 64 Kfar Hanagid, Israel (IL). PAPER, Thierry [FR/FR]; 39, avenue d'Artois, F-75008 Paris (FR). HERZBERG, Max [FR/IL]; Moshav Satariya, 73272 Israel (IL).		<b>(74) Agent:</b> DE BRUIJN, Leendert C.; Nederlandsch Octro- oibureau, Scheveningseweg 82, P.O. Box 29720, NL- 2505 LS The Hague (NL).  <b>(81) Designated States:</b> AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US, Euro- pean patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>

**(54) Title:** METHOD AND APPARATUS FOR DETECTION OF NUCLEIC ACID SEQUENCES**(57) Abstract**

Apparatus for transport of molecules including nucleic acid sequences in a bibulous carrier comprising a dry bibulous carrier defining a capillary transport path which supports the transport of the molecules when contacted with a solution containing the molecules.



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1 Method and apparatus for detection of nucleic acid sequences

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#### FIELD OF THE INVENTION

5 The invention relates to apparatus and methods for  
6 separation of target molecules including target  
7 nucleic acid sequences from oligonucleotides, and  
8 nucleotides and concentration and detection of the  
9 molecules.

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#### BACKGROUND OF THE INVENTION

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15 The use of amplification techniques in a procedure  
16 for detection of a target molecules that include target  
17 nucleic acid sequences is well known in the art.  
18 Typically, this procedure includes enzymatic  
19 amplification of target nucleic acid sequences and  
20 detection of the target molecules by gel  
21 electrophoresis followed by Southern blot procedures.

22 A number of solid phase capture assays have also  
23 been developed to simplify the procedures for detection  
24 of target molecules including nucleic acid sequences.  
25 In these procedures two ligands are typically  
26 incorporated within amplified target nucleic acid  
27 sequences. A first ligand is used to capture, on a  
28 solid matrix, the target molecules that include the  
29 amplified target nucleic acid sequences and a second  
30 ligand is used to detect the target molecules by the  
31 binding of a signal producing reagent to this second  
32 ligand.

33 Solid phase affinity capture assays, however,  
34 require an extended reaction time to capture a high  
35 proportion of target molecules in a reaction mixture  
36 (Sauvaigo et al., Nucleic Acid Research, 1990, Vol. 18,  
37 pp. 3175 - 3182). Furthermore, when capture is mediated  
38 by amplification primers incorporating a solid phase

SUBSTITUTE SHEET

1 affinity ligand, the sensitivity of the assay may be  
2 adversely effected by competition between free primers  
3 and primers incorporated in the target nucleic acid  
4 sequences.

5       The use of chromatography as a separation and  
6 concentration procedure is well known in the art. It  
7 has been reported that whereas DNA molecules are  
8 chromatographically mobile on moistened paper they fail  
9 to migrate when solutions are applied to dry paper  
10 (Bendich et al., Arch. Biochem. Biophys., 1961, 94,  
11 417-423).

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SUMMARY OF THE INVENTION

One object of the present invention is to provide a method and apparatus for capillary transport of molecules including nucleic acid sequences.

Another object of the present invention is to provide a method and apparatus for concentration of target molecules including target nucleic acid sequences in a liquid sample.

A further object of the present invention is to provide a method and apparatus for the separation of target molecules including target nucleic acid sequences from nucleotides and oligonucleotides.

Another object to the present invention is to provide a method for the detection of target molecules including specific nucleic acid sequences.

There is thus provided in accordance with the present invention apparatus for transport of molecules including nucleic acid sequences in a bibulous carrier comprising a dry bibulous carrier defining a capillary transport path which supports the transport of the molecules when contacted with a solution containing the molecules.

In accordance with a preferred embodiment of the invention apparatus for concentration of target molecules in a liquid sample is provided including the dry bibulous carrier wherein the target molecules include target nucleic acid sequences and are transported within the bibulous carrier by capillary action when a portion of the dry bibulous carrier contacts the liquid sample containing the target molecules, and at least one capture reagent immobilized in at least one capture zone on the dry bibulous carrier downstream of a contact portion of the bibulous carrier wherein the at least one capture reagent is capable of capturing the target molecules.

There is also provided in accordance with the

1 present invention apparatus for separation of target  
2 molecules, including target nucleic acid sequences,  
3 from non-target nucleotides and oligonucleotides in a  
4 liquid sample containing the target molecules and the  
5 non-target nucleotides and oligonucleotides comprising,  
6 a vessel containing a compound that binds the non-  
7 target oligonucleotides, and apparatus for transporting  
8 the target molecules from the vessel by capillary  
9 action.

10 In accordance with a preferred embodiment of the  
11 invention the dry bibulous carrier is a nitrocellulose  
12 membrane wherein the absorption sites have been blocked  
13 to facilitate capillary transport of the target  
14 molecules.

15 In accordance with another preferred embodiment  
16 of the invention the dry bibulous carrier is supported  
17 by a rigid frame.

18 In accordance with still another preferred  
19 embodiment of the invention an absorbent pad is fixed  
20 to the dry bibulous carrier downstream from the at  
21 least one capture zone to facilitate capillary  
22 transport of a liquid through the dry bibulous carrier.

23 In accordance with yet another a preferred  
24 embodiment of the invention the absorption sites of the  
25 nitrocellulose membrane are blocked by compounds  
26 selected from a group comprising macromolecules,  
27 detergents and combinations thereof.

28 In accordance with still another preferred  
29 embodiment of the invention the macromolecules include  
30 proteins.

31 In accordance with still a further preferred  
32 embodiment of the invention the at least one capture  
33 reagent includes an antibody to a modified portion of  
34 the target nucleic acid sequences.

35 In accordance with another preferred embodiment of  
36 the invention the at least one capture reagent  
37 includes at least one nucleic acid capture reagent  
38 including nucleic acid probe sequences complementary to

1 at least part of the target nucleic acid sequences.

2 In accordance with still another preferred  
3 embodiment of the invention the nucleic acid probe  
4 sequences include DNA sequences.

5 In accordance with yet another preferred  
6 embodiment of the invention the nucleic acid probe  
7 sequences include RNA sequences.

8 In accordance with a further preferred embodiment  
9 of the invention the target molecules include target  
10 nucleic acid sequences comprising more than 30 base  
11 pairs.

12 In accordance with another preferred embodiment of  
13 the invention wherein the target molecules including  
14 nucleic acid sequences include a nucleic acid product  
15 of an enzymatic amplification reaction and incorporate  
16 at least one pair of oligonucleotide primers.

17 In accordance with still another preferred  
18 embodiment of the invention the at least one pair of  
19 primers include primers for a polymerase chain reaction  
20 (PCR).

21 In accordance with a further preferred embodiment  
22 of the invention the at least one pair of primers  
23 include primers for a ligase chain reaction (LCR).

24 In accordance with yet a further preferred  
25 embodiment of the invention at least a second primer of  
26 the at least one pair of primers includes an  
27 oligonucleotide bearing a ligand which binds to at  
28 least one capture reagent whereby the target molecules  
29 which include the at least one primer bearing the  
30 ligand may be bound to the at least one capture  
31 reagent.

32 In accordance with still a further preferred  
33 embodiment of the invention the ligand which binds to  
34 at the least one capture reagent includes an antigenic  
35 epitope.

36 In accordance with another preferred embodiment of  
37 the invention the ligand which binds to the at least  
38 one capture reagent includes at least one sulfonated

1 cytosine.

2 In accordance with yet another preferred  
3 embodiment of the invention the compound includes gel  
4 filtration particles too large to be transported by the  
5 apparatus for transporting.

6 In accordance with a yet another preferred  
7 embodiment of the invention the non-target  
8 oligonucleotides include oligonucleotide primers not  
9 incorporated in the target nucleic acid sequences.

10 In accordance with a further preferred embodiment  
11 of the invention the compound includes a matrix unable  
12 to be transported by the means for transporting and  
13 wherein the compound hybridizes to the non-target  
14 oligonucleotide.

15 There is also provided in accordance with the  
16 present invention a method for transport of molecules  
17 including nucleic acid sequences in a bibulous carrier  
18 including the steps of, providing a dry bibulous  
19 carrier defining a capillary transport path which  
20 supports the transport of molecules including nucleic  
21 acid sequences, and contacting the dry bibulous carrier  
22 with a solution containing molecules including nucleic  
23 acid sequences.

24 There is additionally provided in accordance with  
25 the present invention a method for concentration of  
26 molecules, including nucleic acid sequences, in a  
27 liquid sample including the steps of, providing a dry  
28 bibulous carrier wherein the molecules are target  
29 molecules including target nucleic acid sequences and  
30 wherein the molecules are transported within the  
31 bibulous carrier by capillary action when a portion of  
32 the dry bibulous carrier contacts the liquid sample  
33 containing the molecules, contacting a portion of the  
34 dry bibulous carrier with the liquid sample containing  
35 the target molecules wherein the dry bibulous carrier,  
36 when wet, defines a liquid transport path which  
37 supports the transport of molecules including nucleic  
38 acid sequences, transporting the target molecules along



1 the liquid transport path, and capturing the target  
2 molecules with at least one capture reagent immobilized  
3 in at least one capture zone on the dry bibulous  
4 carrier downstream of the portion of bibulous carrier  
5 contacting the liquid sample.

6       There is further provided according to the present  
7 invention a method for separation of target molecules,  
8 including target nucleic acid sequences, from non-  
9 target nucleotides and oligonucleotides, in a liquid  
10 sample containing the target molecules and the non-  
11 target nucleotides and oligonucleotides including the  
12 steps of, providing a vessel containing a compound that  
13 binds the non-target nucleotide and oligonucleotide  
14 sequences, adding the liquid sample which includes the  
15 target molecules and the non-target nucleotide and  
16 oligonucleotides, and transporting the target molecules  
17 by capillary action.

18       There is also provided in accordance with the  
19 present invention apparatus for separation of target  
20 molecules, including target nucleic acid sequences,  
21 from non-target nucleotides and oligonucleotides in a  
22 liquid sample containing the target molecules and the  
23 non-target nucleotides and oligonucleotides,  
24 concentration of the target molecules, and detection of  
25 the concentrated target molecules including, a vessel  
26 apparatus defining a plurality of wells including a  
27 first portion of the plurality of wells containing a  
28 compound that binds the non-target oligonucleotides and  
29 wherein the liquid sample may be added to the first  
30 portion of the plurality of wells, a dry bibulous  
31 carrier defining a liquid transport path from the  
32 vessel, that, when wet, supports the transport of the  
33 target molecules, wherein the target molecules are  
34 transported within the bibulous carrier by capillary  
35 action when a contact portion of the dry bibulous  
36 carrier contacts the liquid sample containing the  
37 target molecules, at least one capture reagent capable  
38 of capturing the target molecules wherein the at least

1 one capture reagent is immobilized in at least one  
2 capture zone on the dry bibulous carrier downstream of  
3 the contact portion of the bibulous carrier, and  
4 apparatus for detecting the captured target molecules.  
5 There is further provided in accordance with the  
6 present invention a method for concentration and  
7 detection of target nucleic acid sequences, in a  
8 liquid sample including the steps of, providing a dry  
9 bibulous carrier wherein the target nucleic acid  
10 sequences are transported within the bibulous carrier  
11 by capillary action when a portion of the dry bibulous  
12 carrier contacts the liquid sample containing the  
13 target nucleic acid sequences, contacting a portion of  
14 the dry bibulous carrier with the liquid sample  
15 containing the target nucleic acid sequences wherein  
16 the dry bibulous carrier, when wet, defines a liquid  
17 transport path which supports the transport of the  
18 target nucleic acid sequences, transporting the target  
19 nucleic acid sequences along the liquid transport path  
20 and capturing the target nucleic acid sequences by  
21 hybridization with at least one nucleic acid capture  
22 reagent immobilized in at least one capture zone on the  
23 dry bibulous carrier downstream of the portion of  
24 bibulous carrier contacting the liquid sample. There  
25 is still further provided in accordance with the  
26 present invention apparatus for concentration and  
27 detection of target nucleic acid sequences including,  
28 a vessel apparatus defining a plurality of wells, a  
29 dry bibulous carrier defining a liquid transport path  
30 from the vessel that when wet supports the transport of  
31 the target nucleic acid sequences wherein the target  
32 nucleic acid sequences are transported within the  
33 bibulous carrier by capillary action when a contact  
34 portion of the dry bibulous carrier contacts the liquid  
35 sample containing the target nucleic acid sequences, at  
36 least one nucleic acid capture reagent including  
37 nucleic acid probe sequences for capturing the target  
38 nucleic acid sequences by hybridization and wherein

1 the at least one nucleic acid capture reagent is  
2 immobilized in a capture zone on the dry bibulous  
3 carrier downstream of the contact portion of the  
4 bibulous carrier, and apparatus for detecting the  
5 captured the target nucleic acid sequences.

6 In accordance with a preferred embodiment of the  
7 invention the apparatus for detecting includes a  
8 bibulous carrier upon which target molecules bearing a  
9 ligand which binds to a signal producing reagent are  
10 immobilized, and apparatus for contacting the target  
11 molecules bearing the ligand with the signal producing  
12 reagent to produce a sensible signal indicating the  
13 detection of the target molecules.

14 In accordance with a further preferred embodiment  
15 of the invention the apparatus for detecting includes a  
16 bibulous carrier upon which target molecules bearing a  
17 ligand which binds to a signal producing reagent are  
18 immobilized, and apparatus for contacting the target  
19 molecules bearing the ligand with the signal producing  
20 reagent which react with a color developing reagent to  
21 produce a sensible signal indicating the detection of  
22 the target molecules.

23 In accordance with another preferred embodiment of  
24 the invention the target nucleic acid sequences are  
25 the product of an enzymatic amplification reaction and  
26 incorporate at least one pair of oligonucleotide  
27 primers.

28 In accordance with yet another preferred  
29 embodiment of the invention the non-target  
30 oligonucleotides include oligonucleotide primers not  
31 incorporated in the target nucleic acid sequences.

32 In accordance with still another preferred  
33 embodiment of the invention the at least two sets of  
34 primers include primers for a polymerase chain reaction  
35 (PCR).

36 In accordance with a further preferred embodiment  
37 of the invention the at least one pair of primers  
38 include primers for a ligase chain reaction (LCR).

1 In accordance with still a further preferred  
2 embodiment of the invention a second primer of the at  
3 least one pair of oligonucleotide primers includes a  
4 ligand which binds to the at least one capture reagent  
5 whereby the target molecules that include the ligand  
6 may be bound to the at least one capture reagent.

7 In accordance with yet a further preferred  
8 embodiment of the invention the ligand which binds to  
9 the at least one capture reagent includes an antigenic  
10 epitope.

11 In accordance with another preferred embodiment  
12 of the invention the ligand which binds to the at least  
13 one capture reagent includes at least one sulfonated  
14 cytosine.

15 In accordance with still another preferred  
16 embodiment of the invention a first primer of the at  
17 least one pair of primers includes a ligand which binds  
18 to a signal producing reagent whereby the target  
19 molecules that include the ligand may be detected by  
20 the presence of a signal produced by the signal  
21 producing reagent.

22 In accordance with a further preferred embodiment  
23 of the invention the first primer of the at least one  
24 pair of primers includes a ligand which binds to a  
25 signal producing reagent whereby the target molecules  
26 that include the ligand may be detected by the  
27 presence of a signal produced by the signal producing  
28 reagent after contacting a signal developing reagent.

29 In accordance with yet another preferred  
30 embodiment of the invention the ligand which binds to  
31 the signal producing reagent includes biotinylated  
32 nucleotide sequences. In accordance with a further  
33 preferred embodiment of the invention the signal  
34 producing reagent includes streptavidin linked to  
35 colored latex  
36 beads.

37 In accordance with another preferred embodiment of  
38 the invention the signal produced by the signal

1 producing reagent after contacting the signal  
2 developing reagent includes a streptavidin-alkaline  
3 phosphatase conjugate.

4 In accordance with another preferred embodiment of  
5 the invention the first portion of wells also contains  
6 the signal producing reagent.

7 In accordance with yet a further preferred  
8 embodiment of the invention the plurality of wells  
9 additionally includes a second portion of the wells  
10 containing a washing solution.

11 In accordance with still another preferred  
12 embodiment of the invention the plurality of wells also  
13 includes a third portion of the wells containing a  
14 signal developing reagent solution.

15 In accordance with yet another preferred  
16 embodiment of the invention the dry bibulous carrier  
17 includes at least one strip.

18 In accordance with a further preferred embodiment  
19 of the invention the plurality of wells include a  
20 first portion of wells containing a sample to be tested  
21 for the target nucleic acid sequences.

22 In accordance with another preferred embodiment of  
23 the invention the plurality of wells additionally  
24 include a second portion of the wells containing the  
25 signal producing reagent.

26 In accordance with yet another preferred  
27 embodiment of the invention the plurality of wells  
28 additionally includes a third portion of wells  
29 containing a washing solution.

30 In accordance with still another preferred  
31 embodiment of the invention the plurality of wells  
32 additionally includes a fourth portion of wells  
33 containing a signal developing reagent.

34 In accordance with a further preferred embodiment  
35 of the invention each of the first portion of wells are  
36 adapted to receive the contact portion of each strip to  
37 permit transport of the target molecules to the at  
38 least one capture zone where they are captured.

1 In accordance with still a further preferred  
2 embodiment of the invention each of the second portion  
3 of wells are adapted to receive the contact portion of  
4 each strip for washing the strip to remove non-  
5 specifically captured compounds after immobilization of  
6 the target molecules in the at least one capture zone.

7 In accordance with yet a further preferred  
8 embodiment of the invention each of the third portion  
9 of wells is adapted to receive an entire strip.

10 In accordance with another preferred embodiment of  
11 the invention the apparatus for contacting includes, at  
12 least one of the third portion of wells containing a  
13 signal producing reagent solution, and at least one  
14 strip after immobilization of the target nucleic acid  
15 in the at least one capture zone wherein the entire  
16 strip is in contact with a signal developing reagent  
17 solution permitting contact of the signal developing  
18 reagent with the at least one capture zone.

19 In accordance with yet another preferred  
20 embodiment of the invention each of the first portion  
21 of wells is adapted to receive the contact portion of  
22 each strip to permit transport of the target nucleic  
23 acid sequences to the at least one capture zone where  
24 they are captured.

25 In accordance with still another preferred  
26 embodiment of the invention each of the second portion  
27 of wells is adapted to receive the contact portion of  
28 each strip to permit transport of the signal producing  
29 reagent to the at least one capture zone where the  
30 signal producing reagent is bound to the ligand borne  
31 on the target nucleic acid sequences.

32 In accordance with a further preferred embodiment  
33 of the invention each of the third portion of wells is  
34 adapted to receive the contact portion of each strip  
35 for washing the strip to remove non-specifically  
36 captured compounds after immobilization of the target  
37 nucleic acid sequences in the at least one capture  
38 zone.

1 In accordance with yet a further preferred  
2 embodiment of the invention the apparatus for  
3 contacting includes, at least one of the fourth portion  
4 of wells containing a signal developing reagent, and at  
5 least one strip after immobilization of the target  
6 nucleic acid sequences in the at least one capture  
7 zone wherein the entire strip is in contact with the  
8 signal developing reagent solution permitting contact  
9 of the signal developing reagent with the at least one  
10 capture zone.

11 In accordance with a still further preferred  
12 embodiment of the invention each of the fourth portion  
13 of wells is adapted to receive an entire strip.

14 There is also provided in accordance with the  
15 present invention a method for the detection of a  
16 specific nucleic acid sequence including the steps of,  
17 amplifying by an enzymatic reaction at least a portion  
18 of an original nucleic acid sequence to produce target  
19 molecules including nucleic acid sequences which are  
20 specific to the at least a portion of the original  
21 nucleic acid sequence, separating the target molecules  
22 from non-target nucleotides and oligonucleotides  
23 including the steps of, providing a vessel containing a  
24 substrate that binds the non-target nucleotides and  
25 oligonucleotides, adding a liquid sample which includes  
26 the target molecules and the non-target  
27 nucleotides and oligonucleotides, and transporting the  
28 target molecules by capillary action, concentrating the  
29 target molecules including the steps of, providing a  
30 dry bibulous carrier wherein the target molecules are  
31 transported within the bibulous carrier by capillary  
32 action when a portion of the dry bibulous carrier  
33 contacts the liquid sample containing the target  
34 molecules, contacting a portion of the dry bibulous  
35 carrier with the liquid sample containing the target  
36 nucleic acid sequences wherein the dry bibulous  
37 carrier, when wet, defines a liquid transport path  
38 which supports the transport of the target molecules

1 transporting the target molecules along the liquid  
2 transport path and capturing the target molecules  
3 with at least one capture reagent immobilized in at  
4 least one capture zone on the dry bibulous carrier  
5 downstream of the portion of bibulous carrier  
6 contacting the liquid sample, and detecting the target  
7 molecules by contacting target molecules having a  
8 ligand which binds to a signal producing reagent and  
9 are immobilized on a bibulous carrier with a signal  
10 producing reagent to produce a sensible signal.

11       There is also provided in accordance with the  
12 present invention a method for the detection of a  
13 specific nucleic acid sequence comprising the steps of,  
14 amplifying by an enzymatic reaction at least a portion  
15 of an original nucleic acid sequence to produce target  
16 nucleic acid sequences which are specific to the at  
17 least a portion of the original nucleic acid sequence,  
18 providing a liquid sample which includes the target  
19 nucleic acid sequences, transporting the target nucleic  
20 acid sequences by capillary action, concentrating the  
21 target nucleic acid sequences including the steps of  
22 providing a dry bibulous carrier wherein the target  
23 nucleic acid sequences are transported within the  
24 bibulous carrier by capillary action when a portion of  
25 the dry bibulous carrier contacts the liquid sample  
26 containing the target nucleic acid sequences,  
27 contacting a portion of the dry bibulous carrier with  
28 the liquid sample containing the target nucleic acid  
29 sequences wherein the dry bibulous carrier, when wet,  
30 defines a liquid transport path which supports the  
31 transport of the target nucleic acid sequences, and  
32 transporting the target nucleic acid sequences along  
33 the liquid transport path, capturing the target  
34 nucleic acid sequences with at least one nucleic acid  
35 capture reagent immobilized in at least one capture  
36 zone on the dry bibulous carrier downstream of the  
37 portion of bibulous carrier contacting the liquid  
38 sample and detecting the target nucleic acid sequences



1 by contacting target nucleic acid sequences having a  
2 ligand which binds to a signal producing reagent and  
3 are immobilized on a bibulous carrier with a signal  
4 developing reagent to produce a sensible signal.

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## 1 BRIEF DESCRIPTION OF THE DRAWINGS

2 The present invention will be understood and  
3 appreciated more fully from the following detailed  
4 description taken in conjunction with the drawings in  
5 which:

6 Fig. 1 is a front view pictorial illustration of  
7 apparatus for separation of a target nucleic acid  
8 sequences from non-target nucleotides and  
9 oligonucleotides in a liquid sample, concentration of  
10 the target nucleic acid sequences, and detection of the  
11 concentrated target nucleic acid sequences constructed  
12 and operative in accordance with the present invention  
13 and shown before use;

14 Fig. 2 is a front view pictorial illustration of  
15 the apparatus of Fig. 1 shown during use;

16 Fig 3 is a front pictorial view of an alternative  
17 embodiment of the apparatus of Fig. 1 shown before use;  
18 and

19 Fig. 4 is a front pictorial view illustration of  
20 the apparatus of Fig. 3 shown during use.

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2 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS  
3

4 Reference is now made to Figs. 1 - 4 which  
5 illustrates apparatus 10 for separation of a target  
6 molecules including target nucleic acid sequences from  
7 non-target nucleotides and oligonucleotides in a liquid  
8 sample, concentration of the target molecules, and  
9 detection of the concentrated target molecules  
10 constructed and operative in accordance with a  
11 preferred embodiment of the present invention.

12 Apparatus 10 includes vessel apparatus 12  
13 fabricated from a non-porous material such as  
14 polystyrene and including one or more of a plurality of  
15 wells such as wells 14, 16 and 18. The wells, such as  
16 wells 14, 16, and 18, are approximately 1 cm in length,  
17 0.5 cm in width, and 2.5 cm in depth, and are sized to  
18 receive the a contact portion 20 of a strip 22.

19 The strip 22 includes a bibulous carrier 24  
20 typically embodied in a mylered nitrocellulose membrane  
21 approximately 3.0 cm in length and 0.5 cm in width and  
22 having a pore size of 3 - 5 microns which may be  
23 surrounded by a support frame 26. The support frame 26  
24 is fabricated from a non-porous material such as  
25 polystyrene, and bibulous carrier 24 may be mounted in  
26 frame 22 by any convenient means such as gluing. An  
27 absorbent pad 27 approximately 2 cm in length and 0.5  
28 cm in width, fabricated from an absorbent material such  
29 as Whatman 3MM paper (commercially available from  
30 Whatman, Maidstone, U.K.) is attached to the end of the  
31 strip 22 opposite the contact portion 20 by any  
32 convenient means such as gluing. The end of strip 22  
33 is also attached to a handle 28 by any convenient means  
34 such as gluing. The handle 28 is fabricated from a non-  
35 porous material such as polystyrene. At least one strip  
36 22 is attached to the handle 28 to form a test member  
37 30.

38 A single capture reagent is typically immobilized

1 on the bibulous carrier 24 in the central area of the  
2 bibulous carrier, to form a capture zone 32. Although a  
3 single capture reagent is typically employed, multiple  
4 capture reagents may be used to form multiple capture  
5 zones on a single bibulous carrier.

6 The single capture reagent, typically an anti-  
7 sulfonated DNA antibody or a nucleic acid  
8 complementary to at least part of the target nucleic  
9 acid sequence, is typically immobilized by absorption  
10 on the nitrocellulose membrane.

11 Wells 14 typically contain an enzymatic  
12 amplification reaction mixture. In addition, when the  
13 capture reagent is an anti-sulfonated DNA antibody the  
14 wells 14 also typically contain gel filtration  
15 particles (not shown), typically Sephadex G-100  
16 (Pharmacia, Uppsala, Sweden) gel filtration particles.  
17 The gel filtration particles are sized to be too large  
18 to be transported by capillary action in the bibulous  
19 carrier 24.

20 The procedure used to detect specific nucleic acid  
21 sequences using apparatus 10 typically includes the  
22 enzymatic amplification of the specific nucleic acid  
23 sequence using Polymerase Chain Reaction (PCR) or  
24 Ligase Chain Reaction (LCR) employing at least one pair  
25 of primers. At least a first primer of the at least one  
26 pair of primers of these reactions bears an affinity  
27 ligand, typically biotin, which binds to a signal  
28 producing reagent, typically a streptavidin alkaline  
29 phosphatase conjugate. In addition, when the capture  
30 reagent is an anti-sulfonated DNA antibody at least one  
31 second primer of the at least one pair of primers for  
32 the enzymatic amplification bears an affinity ligand,  
33 typically a sulfonated cytosine, which is bound by the  
34 capture reagent of the capture zone 32. After a number  
35 of amplification cycles, typically between 1 and 50  
36 cycles, an aliquot of a reaction mixture is assayed  
37 using apparatus 10.

38 When the capture reagent is an anti-sulfonated DNA

1 antibody, an aliquot of the reaction mixture containing  
2 target nucleic acid sequences, oligonucleotide primers,  
3 and nucleotides, typically between 1 and 20  $\mu$ l is added  
4 to well 14. Approximately 30  $\mu$ l of a solution  
5 containing a signal producing reagent, typically  
6 streptavidin alkaline phosphatase conjugate in a TPG  
7 running buffer (0.3% Tween 20 and 1% gelatin in PBS),  
8 is also added to well 14 and the contact portion 20 of  
9 strip 22 is placed in well 14 in contact with the  
10 reaction mixture. The reaction mixture containing the  
11 target molecules including the nucleic acid sequences  
12 is carried through the bibulous carrier 24 by  
13 capillary transport, past the capture zone 32 where  
14 the target molecules are captured by the capture  
15 reagent, to the absorbent pad 27.

16 After about 10 minutes most of the molecules that  
17 include labeled nucleic acid sequences (typically more  
18 than 80% of the labeled molecules) are captured in the  
19 capture zone 32. The contact portion 20 of the strip 22  
20 is then removed from the well 14 and placed in the well  
21 16.

22 The well 16 typically contains about 50  $\mu$ l of TP  
23 buffer (0.3% tween in PBS) which is carried through  
24 the bibulous carrier 24 to the capture zone to remove  
25 non-specifically captured compounds which may interfere  
26 with the detection of the target nucleic acid sequence.  
27 After about 10 minutes strip 22 is removed from well 16  
28 and immersed in well 18.

29 Well 18 contains about 300  $\mu$ l of signal developing  
30 reagent solution, typically a Chemiprobe<sup>tm</sup> solution  
31 containing the chromogenic substrate, BCIP/NBT,  
32 commercially available from Organics Ltd., Yavne  
33 Israel). This solution covers the capture zone 32. The  
34 signal producing reagent, alkaline phosphatase, which  
35 is attached to the labeled molecules in the capture  
36 zone 32 then converts the chromogenic substrate to a  
37 precipitable color which is a sensible signal  
38 indicating detection of the target nucleic acid

1 sequences.

2       When the capture reagent is a nucleic acid  
3 complementary to at least part of the target nucleic  
4 acid sequence an aliquot of the reaction mixture is  
5 typically diluted with a hybridization solution  
6 typically composed of 0.6M NaCl, 20mM phosphate buffer,  
7 pH 7.5, 0.02% Ficoll 400 (Sigma, St. Louis, MO, USA),  
8 0.02% gelatin and 1% PVP. The sample is then typically  
9 boiled and chilled immediately and an aliquot of each  
10 solution transferred to the wells 14 of the apparatus  
11 12. The contact portion 20 of each strip 22 is then  
12 typically brought into contact with the solution in the  
13 wells 14.

14       Apparatus 10 is then typically placed in a humid  
15 incubator for approximately 25 minutes and the solution  
16 allowed to migrate through the nitrocellulose strips  
17 forming the bibulous carrier 24. The solution  
18 containing the target molecules including the nucleic  
19 acid sequences is carried through the bibulous carrier  
20 24 by capillary transport to the absorbent pad 27 and  
21 past the capture zone 32 where the target molecules are  
22 captured by the nucleic acid complementary to the  
23 target nucleic acid sequence.

24       The strips 22 are then typically transferred to  
25 wells 16 containing streptavidin alkaline phosphatase  
26 conjugate. The strips 22 are then typically transferred  
27 to wells 18 containing a solution including 150  $\mu$ l of  
28 0.3% Tween 20 in PBS and the contact portion 20 of the  
29 strip 22 was brought into contact with the solution for  
30 approximately 15 minutes.

31       Finally the strips 22 are then typically  
32 completely immersed in a ChemiProbe<sup>tm</sup> BCIP/NBT solution  
33 in a set of wells not shown in the figures for  
34 approximately 20 minutes to provide a substrate for a  
35 chromogenic reaction. A blue colored signal in the  
36 capture zone 32 of strip 22 indicating the presence the  
37 target molecules.

38       As can be seen in Figs. 3 and 4 more than one

1 strip 22 can be attached to handle 28 to permit more  
2 that one assay to carried out at the same time.

3 Reference is now be made to the following examples  
4 which, together with Figs. 1 - 4 illustrate the  
5 invention.

6

7

8

9

#### EXAMPLE 1

#### TRANSPORT AND CONCENTRATION OF DNA ON NITROCELLULOSE

11

##### 12 a) Sequence synthesis and labeling of primers

13 Primers were selected in the gene of HIV-1 and had the  
14 following sequences:

15 Primer 3

16 5'TGGGAAGTTCAATTAGGAATACCAC

17 Primer 3'5'TGGGAAGTTCAATTAGGAATA

18

19 Primer 4

20 5'CCTACATACAAATCATCCATGTATTC

21

22 The primers were synthesized on Applied Biosystems  
23 380A DNA Synthesizer (Applied Biosystems, Hayward, CA,  
24 USA) and purified using OPC rapid purification  
25 cartridges (Applied Biosystems, CA, USA).

26

##### 27 Primer sulfonation

28 The primer 3' was synthesized with a 13 mere  
29 Polycytosine Tail at the 5' end. These primer was then  
30 sulfonated according to the protocol described in the  
31 ChemiProbe<sup>tm</sup> kit (commercially available from Orgenics  
32 Ltd.).

33 100 µl of C Tail primer (0.5 mg/ml) was mixed with  
34 50 µl of solution A of the ChemiProbe<sup>tm</sup> Kit (4M sodium  
35 bisulfite) and 12.5 µl of solution B of the  
36 ChemiProbe<sup>tm</sup> Kit (1M methoxyamine) and incubated  
37 overnight at 20°C. Sulfonated oligonucleotides were  
38 then desalted by centrifuging through a 2 ml bed of

1 Sephadex G-50 spin column.  
2  
3 Primer biotinylation  
4 Primer 4 was synthesized in the 5' end with a 12 mere  
5 polycytosine in which 4 cytosine nucleotides were  
6 replaced by N<sup>4</sup>-LCA-5-methyldeoxycytidine (American  
7 Bionetics, Hayward, CA, USA) as follows CCCCCCCCCCCC,  
8 where C indicates the modified cytosine. These  
9 oligonucleotides were purified by acrylamide gel  
10 according to the procedures described by Maniatis, T.  
11 et al., Molecular cloning: a laboratory manual, 1989, p  
12 646, Cold Spring Harbor Laboratory, Cold Spring Harbor,  
13 N.Y. the teachings of which are herein incorporated by  
14 reference.

15 The purified oligonucleotides were then  
16 biotinylated according to the following procedure:

17 10 nmole of desiccated primers were dissolved in  
18 50 µl of 100 mM Borate Buffer and added to 50 µl of  
19 dimethyl formamide (DMF) containing 0.1 mg of biotin N  
20 Hydroxy succinimide (Pierce, Rockford, Ill. USA). This  
21 solution was then incubated overnight at 20°C and then  
22 purified through a Nensorb 20 column (Du Pont Company,  
23 Wilmington, DE, USA) according to the instructions of  
24 the supplier. The primers were then concentrated by  
25 evaporation and resuspended with water to the original  
26 concentration.

27

28 b) Amplification of the HIV sequence 100 µl of a  
29 mixture containing 1 µg of extracted DNA from a  
30 positive HIV sample (extraction procedure according to  
31 Edwards et al., The Journal of Pediatrics, 1989, vol.  
32 45, pp 200-203) the teachings of which are herein  
33 incorporated by reference. 100 pmole of each primer P3  
34 and P4, 0.25 mM of the four deoxynucleotide  
35 triphosphate (dNTP), 10 µl 10X Taq Buffer (Promega ,  
36 Madison, Wisconsin. USA) and 2.5 U of Taq polymerase  
37 (Promega) was amplified under the following conditions  
38 on a programmable Grant (Cambridge, U.K.) water bath.



1 A first DNA denaturing step of 5 minutes at 94°C  
2 was followed by 30 cycles of 1 minute denaturing at  
3 94°C. 1 minute DNA annealing at 52°C and 1 minute DNA  
4 elongation at 72°C. The amplification was ended with a  
5 seven minute elongation step at 72°C.

6 A second amplification was performed for 20 cycles  
7 under the same conditions as the first amplification  
8 but using the labeled biotinylated and sulfonated  
9 primers described above. The DNA template employed was  
10 1 µl of the first PCR mixture diluted in 100 µl of a  
11 mixture containing 100 pmoles of each labeled primer,  
12 0.25 mM of the four deoxynucleotide triphosphate, 10 µl  
13 of 10X Taq buffer (Promega) and 2.5 U of Taq polymerase  
14 (Promega). Primers were excluded from the PCR Product  
15 by mixing 100 µl of the reaction mixture with 60 µl of  
16 polyethylene glycol (PEG) 4000 (Sigma, St. Louis. MO,  
17 USA) in 2.5 M NaCl solution. This mixture was then  
18 incubated for one hour at 4°C. Then, after 10 minutes  
19 of centrifugation at 10,000 xg at 4°C the supernatant  
20 was discarded and the pellet was resuspended in 100 µl  
21 of water.

22

23 c) Preparation of nitrocellulose backed strips

24 1. Mylered Nitrocellulose (pore size 3 µ)  
25 (Schleicher & Schuell, Dussel, Germany) were cut into  
26 lengths of 0.5 x 3.0 cm to form the bibulous carrier 24  
27 of the apparatus of Figs. 1 - 4. The bibulous carriers  
28 24 formed strips 22. One microliter of purified mouse  
29 monoclonal anti-modified DNA (2 mg/ml), commercially  
30 available from Orgenics Ltd., catalog no. 10793010,  
31 supplemented with 1% sucrose in phosphate buffered  
32 saline (PBS) was embedded in the middle of the  
33 nitrocellulose strips in a horizontal line to form the  
34 capture zone 32. The strips were then air dried for 1  
35 hour at 37°C.

36 Free absorption sites were then blocked by  
37 incubating the strips for 2 hours in a solution of 1%  
38 gelatin (Norland Products Inc., New Brunswick Canada),

1 and 0.05% Tween 20 (Sigma) in PBS. The nitrocellulose  
2 strips were then briefly washed in water, dried for one  
3 hour in an incubator at 37°C and stored under  
4 desiccation for at least four months. A square of 0.5x  
5 2 cm of Whatman 3MM paper was attached to the top of  
6 the strip to serve as an absorbent pad 27.

7 2. Mylered nitrocellulose lengths were prepared as  
8 above but without the blocking step.

9

10 d) Transport and concentration of the DNA

11 The PCR reaction mixture was diluted ten fold in  
12 either TGP running buffer (0.30% Tween 20 and 1%  
13 gelatin in PBS), or PBS. 30 µl of each solution were  
14 then transferred to wells similar to the wells of  
15 apparatus 12 shown in Figs. 1 - 4 and the contact  
16 portion 20 of each strip 22 was brought into contact  
17 with the solutions.

18 The solution was allowed to migrate through the  
19 nitrocellulose strips forming the bibulous carrier 24  
20 at room temperature for 10 minutes. The strips 22 were  
21 then covered completely by a solution of streptavidin  
22 alkaline phosphatase conjugate (Enzymatix, Cambridge,  
23 U.K.) diluted 1:2,500. After a 10 minute incubation at  
24 room temperature the strips were washed briefly with  
25 water and then covered by BCIP/NBT ChemiProbe™  
26 solution (Orgenics Ltd.). After 5 minutes the strips  
27 were briefly washed with water and inspected. The color  
28 was then stabilized by a brief washing in ethanol and  
29 then dried at room temperature. A strip 22 was  
30 considered positive for HIV if a purple line appeared  
31 in the capture zone.

32 Running the HIV product of PCR amplification on  
33 nitrocellulose strips using PBS as a buffer wherein  
34 the absorption sites of the nitrocellulose strips were  
35 not blocked failed to produce a positive reaction. The  
36 strips 22, however, in which the free absorption sites  
37 of the nitrocellulose were blocked by gelatin solution  
38 produced a visible signal when PBS was used as a

1 running buffer. In addition, the strips 22, wherein the  
2 absorption sites were not blocked prior to their  
3 contact with PCR reaction mixture solutions produced a  
4 visible signal when the TGP running buffer was used.  
5 The strongest signal was obtained when both a blocked  
6 strip and the TGP running buffer were used.

7        These results indicate that amplified nucleic acid  
8 sequences can migrate by capillary movement through  
9 nitrocellulose strips wherein the absorption sites of  
10 the nitrocellulose are blocked either prior to or  
11 during the capillary transport of the nucleic acid  
12 sequences. Moreover these results also indicate that  
13 amplified DNA in a solution may be concentrated by  
14 contacting blocked nitrocellulose strips at a contact  
15 point with a solution containing amplified DNA and  
16 capturing the amplified DNA at an appropriate capture  
17 site on the nitrocellulose strip downstream of the  
18 contact point.

19

20

21

22

#### EXAMPLE 2

#### 23 TRANSPORT AND CONCENTRATION OF GENOMIC AND PLASMID DNA 24 ON NITROCELLULOSE

25

26        Human Placenta DNA (Sigma), CasKi cells DNA and  
27 Bluescript plasmid DNA were prepared and sulfonated as  
28 described by Nur et al. (Ann. Biol. Clin., 1989, 47,  
29 601 - 606) with each molecule of CasKi cell DNA or  
30 Human Placental DNA having about  $10^{15}$  base pairs. HIV  
31 specific PCR products were amplified with one primer  
32 being sulfonated another primer being biotinylated,  
33 thus double labeling the PCR products as described in  
34 Example 1. The nitrocellulose strips 22 having blocked  
35 absorption sites were also prepared as described in  
36 Example 1.

37        One  $\mu$ l of a 20  $\mu$ g/ml solution of each of the three  
38 types of DNA (either sulfonated or unsulfonated) was

1 added to 20  $\mu$ l of TGP running buffer. The DNA solution  
2 was loaded into wells and the contact portion 20 of the  
3 strips 22 brought into contact with this solution.  
4 After 10 minutes the strips were removed from the DNA  
5 solution and transferred to other wells where the  
6 contact portion 20 of the strips 22 was brought into  
7 contact with double label PCR product (diluted 1:20  
8 from the HIV PCR reaction mixture solution of Example  
9 1) and streptavidin alkaline phosphatase conjugate  
10 (Enzymatix, Cambridge, U.K.) diluted 1:2,500 in TGP  
11 running buffer. After 10 minutes of contact with the  
12 double label DNA product the strips 22 were washed for  
13 10 minutes by contacting the contact portion of strips  
14 22 with a washing solution of TGP buffer. Finally, the  
15 strips 22 were immersed in a ChemiProbe<sup>tm</sup> BCIP/NBT  
16 solution (commercially available from Orgenics Ltd.)  
17 for a 5 minute incubation period as described in  
18 Example 1.

19 It was found that all three types of DNA,  
20 Placental DNA, CasKi cell DNA and Bluescript plasmid  
21 DNA, when sulfonated completely prevent the development  
22 of a visible signal in the capture zone 32. In contrast  
23 to these results, solutions containing the same DNA,  
24 but where the DNA was not sulfonated failed to inhibit  
25 the signal. These results indicate that both genomic  
26 DNA and plasmid DNA can be transported by capillary  
27 movement of a liquid through a nitrocellulose carrier  
28 and that this DNA can be concentrated at an appropriate  
29 capture site on the nitrocellulose strip.

30 The above results also suggest that the presence  
31 of target DNA in a sample can be detected by the  
32 reduction in signal produced by the double label PCR  
33 product when target DNA is sulfonated and bound to  
34 the capture zone 32 before capturing the double label  
35 DNA as described above.

36

37

38

### EXAMPLE 3

#### COMPARISON OF DETECTION SYSTEMS

1 Primers were selected in the E6 gene of the HPV  
2 genome and were consensus primers for HPV 16, HPV 18  
3 and HPV 33 described in Israel Patent Application No.  
4 097226 the teachings of which are herein incorporated  
5 by reference. These primers had the following  
6 sequences:

7

8 Primer h15'AAGGGAGTAACCGAAATCGGT

9 Primer h25'ATAATGTCTATATTCACTAATT

10

11 The primer synthesis and labeling procedure was  
12 described in Example 1. Primer h1 was sulfonated and  
13 Primer h2 biotinylated according to these procedures.

14

15 Amplification and labeling of HPV DNA SEQUENCE

16

17 100  $\mu$ l of reaction mixture containing 100 pmole of  
18 labeled or unlabeled primers, 1  $\mu$ g of DNA extracted  
19 from cervical biopsies according to the instructions of  
20 the HybriComb<sup>tm</sup> HPV kit (commercially available from  
21 Organics Ltd.), 0.25 mM of deoxynucleotide triphosphate  
22 (dNTP), 10 $\mu$ l 10X Taq buffer (commercially available  
23 from Promega), and 2.5 units of Taq polymerase  
24 (commercially available from Promega). The  
25 thermocycling of the mixture was performed with a Grant  
26 programmable water bath.

27 A first PCR step was performed using the unlabeled  
28 primers. Each amplification cycle consisted of: DNA  
29 denaturing for 1 minute at 94°C, annealing step 1  
30 minute at 55 °C, and DNA extension step for 1 minute at  
31 72°C. The amplification reaction was terminated by 5  
32 minutes of extension at 72°C after 20 cycles. A second  
33 PCR step using labeled primers was performed according  
34 to the following procedure. One  $\mu$ l of the first  
35 reaction mixture was added to each of six replicates  
36 containing 100  $\mu$ l of reaction mixture identical to  
37 that of the first PCR reaction (except that labeled  
38 rather than non-label primers were used). Each

1 replicate was amplified for either 0, 10, 20, 25, or 30  
2 cycles and then stored at 4°C.

3

4 Detection of the PCR product

5 1. Detection by ethidium bromide - EtdBr.

6 After amplification, 10 µl of the PCR mixture was  
7 electrophoresed on 8% non-denaturing (TAE) Tris-acetic  
8 acid buffer polyacrylamide gel and electrophoresed for  
9 1 hour at 50 mA. Gels were submerged for 15 min. in 10  
10 µg/l of ethidium bromide (EtdBr) and DNA was visualized  
11 by UV light.

12

13 2. Detection by Southern blot.

14 After separation by electrophoresis the migrated  
15 PCR fragments were electroblotted onto Hybond-N  
16 membrane (commercially available from Amersham, Bucks,  
17 U.K.) using TAE buffer as the transfer buffer in a  
18 Trans Blot Cell (Commercially available from Bio-Rad,  
19 Richmond, CA, USA) for 3 hours at 1.5 Amp. The membrane  
20 was then air dried and baked for 2 hours at 80°C.

21 Visualization of the biotinylated label was  
22 performed as follows: The membrane was blocked by PBS  
23 supplemented with 1-light (Tropix, MA, USA) and 0.1%  
24 Tween 20. The nylon membrane was incubated for 1 hour  
25 in the same blocker supplemented with streptavidin  
26 alkaline phosphatase conjugate diluted 1:2500 and then  
27 washed by a solution containing 0.1% Tween 20 in PBS.  
28 Finally, the membrane immersed in a ChemiProbe<sup>tm</sup>  
29 BCIP/NBT chromogenic solution for 30 minutes and the  
30 excess chromogen rinsed with water.

31

32 3. Detection by solid support capture (dip-stick)  
33 assay.

34 Non-bibulous impact polystyrene (commercially  
35 available from Orgenics Ltd.) was used as a solid  
36 support for a dip-stick type capture assay.

37 Preparation of the dip-stick. One microliter of a  
38 solution of 2 mg/ml purified mouse monoclonal anti-

1 modified DNA in PBS was applied to the lower portion of  
2 the dip-stick and then dried for 1 hour at 37°C. The  
3 unbound sites were blocked by dipping the dip-stick  
4 into a solution of 1% gelatin and 0.05% Tween 20 for 1  
5 hour. The dip-sticks were then washed for 2 - 5 seconds  
6 in water and dried at 37°C for 1 hour.

7 The assay:

8       5  $\mu$ l of a reaction mixture solution from each of  
9 the second PCR cycle groups was added to 45  $\mu$ l of TGP  
10 running buffer containing streptavidin alkaline  
11 phosphatase conjugate (1:200). The solutions were  
12 placed in wells and the dip-stick was dipped into the  
13 solutions. After 30 minutes incubation the dip-sticks  
14 were washed in PBS and dipped in BCIP/NBT solution for  
15 20 minutes. The reaction was terminated by washing the  
16 dip-sticks in water.

17

18 4. Detection by Capillary DNA Concentration Assay  
19 (CDCA).

20       3  $\mu$ l of each of reaction mixture solution from  
21 each of the second PCR cycle groups was added to wells  
22 containing 30  $\mu$ l of solution containing streptavidin  
23 alkaline phosphatase conjugate diluted 1:2,500 in TGP  
24 running buffer. Nitrocellulose strips were prepared as  
25 in Example 1. The contact portion 20 of the strips 22  
26 were brought into contact with the solution in the  
27 wells for 10 minutes. The contact portion of the strips  
28 22 were then brought into contact for 10 minutes with  
29 wells containing 50  $\mu$ l of washing solution (TP buffer).  
30 Finally, the strips 22 were completely immersed in a  
31 ChemiProbe<sup>tm</sup> BCIP/NBT solution for 5 minutes to provide  
32 a substrate for a chromogenic reaction.

33       The results of the above procedures are present in  
34 Table 1 which indicates the detection limit in  
35 relation to the number of PCR cycles for the assays  
36 described above - EtdBr, Southern blot, solid support  
37 capture assay and CDCA.

38

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18 ± = threshold levels  
 19 - = definite negative  
 20 + = definite positive

21  
 22  
 23 As can be seen from Table 1 the sensitivity of the  
 24 dip-stick test is similar to that of the EtdBr  
 25 fluorescence test, both of which are less sensitive  
 26 than the Southern Blot technique. The CDCA was seen to  
 27 be at least as sensitive as the Southern blot  
 28 technique.

#### 31 EXAMPLE 4

#### 32 EFFECT OF PRIMER ELIMINATION AFTER AMPLIFICATION ON THE

#### 33 SENSITIVITY OF THE CDCA PROCEDURE

34 Specific HIV sequences were amplified from a  
 35 positive HIV sample in a 100µl reaction mixture for 20  
 36 PCR cycles using 100 pmole of unlabeled Primer 3 and  
 37 Primer 4 as described in Example 1. The second  
 38 amplification was performed under the same conditions



1 as the first amplification but with labeled primers and  
2 for 2, 4, 6, 8, 10, and 20 cycles. The template for the  
3 second PCR amplification was 1  $\mu$ l of the first PCR  
4 mixture diluted in 100  $\mu$ l of reaction mixture  
5 containing 100 pmoles of each labeled primer, 0.25 mM  
6 of the four deoxynucleotide triphosphate, 10  $\mu$ l of 10X  
7 Taq buffer (Promega) and 2.5 U of Taq polymerase  
8 (Promega). For each PCR amplification cycle number  
9 group, 4 aliquots of 100  $\mu$ l of PCR reaction mixture  
10 were tested, one for each assay .

11

## 12 Assay-1.

13 The first assay was the CDCA system described in  
14 Example 3. From each PCR amplification cycle number  
15 group, 3  $\mu$ l of reaction mixture were added to wells  
16 containing 30  $\mu$ l streptavidin alkaline phosphatase in  
17 TGP running buffer and the CDCA was performed as  
18 described in Example 3.

19

## 20 Assay-2

21 In the second assay the PCR reaction mixture was  
22 treated with PEG to remove primers before running the  
23 CDCA. Primers of each PCR amplification cycle number  
24 group were excluded using a PEG solution as described  
25 in Example 1. 3  $\mu$ l of the PEG treated PCR amplification  
26 mixture was added to 30  $\mu$ l of TGP running buffer and  
27 the assay then performed as in Example 3.

28

## 29 Assay-3

30 In the third assay primers in the PCR reaction  
31 mixture were excluded by Sephadex G-100 prior to CDCA.  
32 Primers of each of the PCR amplification cycle number  
33 group were excluded by Sephadex G-100 as follows. 0.5  
34 ml of Tris EDTA buffer (TE) in Sephadex G-100  
35 (Pharmacia) was transferred to a well, excess TE was  
36 absorbed by filter paper. 15  $\mu$ l of each PCR reaction  
37 mixture solution was diluted 1:1 with TGP running  
38 buffer and the mixture placed directly in the bottom of

1 the well.

2 The contact portion 20 of a strip 22, including a  
3 strip of nitrocellulose wherein the absorption sites  
4 are blocked was prepared as in Example 1, was brought  
5 into contact with the upper side of the Sephadex G-100  
6 for 25 minutes. The contact portion of the strip 22 was  
7 then brought into contact for 10 minutes with  
8 streptavidin alkaline phosphates conjugate diluted  
9 1:2,500 in TGP running buffer in a well, then washed  
10 and visualized according to the procedure of Example 1.

11

#### 12 Assay-4

13 In the fourth assay primers were removed from the  
14 PCR reaction mixture prior to the CDCA by hybridization  
15 of the primers to complementary oligonucleotide  
16 sequences bound to a compound. Primers of each PCR  
17 amplification cycle number group were trapped by being  
18 brought into contact with beads coated with  
19 oligonucleotides having sequences complementary to the  
20 sequences of the primers to be trapped.

21

22 a) Preparation of the trapping system. Streptavidin  
23 was bound to styrene/vinyl carboxylic acid beads (5  $\mu$ m  
24 in diameter commercially available from Bangs  
25 Laboratories, Inc. Carmel, IN, USA) according to the  
26 principles of Woodward, R.B. and Elofson, R.A. (1961).  
27 J. Amer. Chem. Soc. 83, 1007-1010 under conditions  
28 described in Israel Patent Application 098452, the  
29 teachings of which are herein incorporated by  
30 reference. The complementary oligonucleotide sequence,  
31 5' TATTCCTAATTGAACTCAA was synthesized and  
32 biotinylated as described in Example 1.

33 The oligonucleotide was bound to the beads by the  
34 following procedure. 100  $\mu$ l of 1% coated beads were  
35 mixed 1:1 with a solution of 1mg/ml of biotinylated  
36 oligonucleotide. The solution was incubated for 3 hours  
37 at 30°C. The unbound oligonucleotide was washed in PBS  
38 and kept in a solution of 1% gelatin in PBS.

1  
 2 b) The assay procedure 3  $\mu$ l of each PCR amplification  
 3 cycle number group was added to wells containing 30  $\mu$ l  
 4 of a solution containing 0.50% complementary  
 5 oligonucleotide coated beads and streptavidin alkaline  
 6 phosphatase conjugate (diluted 1:500) in TGP buffer and  
 7 allowed to incubate for 10 minutes.

8 A contact portion 20 of strip 22, including a  
 9 nitrocellulose strip wherein the absorbent sites were  
 10 blocked and was prepared as in Example 1, is then  
 11 brought into contact with the incubated solution for 10  
 12 minutes. The strip 22 was then washed and the signal  
 13 developed as in Example 3.

14 Table 2 shows the effect of elimination of primers  
 15 after amplification on the sensitivity of the CDCA.

16

17	Table 2						
18	Detection Limit of Assays 1-4						
19	Number of PCR cycles						
20	0	2	4	6	8	10	20
21	System						
22	=====						
23	Assay 1					+	+
24							
25	Assay 2	+	+	+	+	+	+
26							
27	Assay 3		+	+	+	+	+
28							
29	Assay 4			+	+	+	+
30							
31	=====						

32

33 + = detection of the HIV DNA sequences.

34 As can be seen from Table 2 untreated PCR solution  
 35 fails to provide a visible signal in the CDCA assay  
 36 even after 8 cycles of amplification. Only after some  
 37 10 cycles does a positive response appear. Elimination  
 38 of the primers after amplification by a separation

1 stage or during the test enables the detection of  
2 target nucleic acid sequences after only 2 - 6 PCR  
3 cycles. Elimination of primers by each technique has  
4 been confirmed by gel electrophoresis and visualization  
5 by EtdBr (data not shown).

6

7

#### EXAMPLE 5

8

#### 9 DETECTION OF HPV SEQUENCES IN CLINICAL SAMPLES BY 10 HYBRIDIZATION IN SOLUTION

11

12 Preparation of the probe. A single stranded HPV sequence  
13 was prepared by asymmetric PCR amplification using the  
14 HPV primer h1 described in Example 3. The following  
15 conditions for amplification were employed. 10 ng of  
16 non-labeled HPV PCR product prepared as described in  
17 example 3 was used as a template and only one primer h1  
18 was used for amplification. 50 PCR cycles were  
19 performed as described in Example 3.

20 The single stranded product was then sulfonated  
21 for one hour at 30°C and was then desalted by using  
22 Sephadex G-50 as described in the instructions for the  
23 use of the ChemiProbe<sup>™</sup> kit (Organics, Ltd.)

24

#### 25 Amplification of the HPV Sequence

26 The HPV sequences were amplified from a clinical  
27 sample by two methods: A) using biotinylated h2 primers  
28 and non labeled h1 primers and B) using biotinylated  
29 h2 primers and sulfonated h1 primers. For both methods  
30 PCR was performed as described in Example 3 for 35  
31 cycles.

32

#### 33 Hybridization

34 5 µl of the PCR reaction mixture solution of  
35 method A (after 35 cycles) was added to 95 µl of a  
36 hybridization solution containing 0.66M NaCl, 65mM  
37 sodium citrate, 0.3 mM EDTA, 0.1M phosphate buffer pH  
38 6.6, 0.02% Ficoll<sup>™</sup>, 0.2% Polyvinylpyrrolidone, 0.5%

1 Polyethylglycol, 0.12% bovine serum albumin, and 100 ng  
2 of a sulfonated probe described above. The solution was  
3 then heated for 5 minutes at 95°C and cooled  
4 immediately. Hybridization was performed for 45 minutes  
5 at 65°C.

6

7 Capture by CDCA

8 3  $\mu$ l of the hybridization mixture after completion  
9 of the hybridization or 0.3  $\mu$ l of PCR reaction mixture  
10 solution from method B were added to wells containing  
11 30  $\mu$ l of streptavidin alkaline phosphatase in TGP  
12 running buffer. A contact portion 20 of strip 22,  
13 including a nitrocellulose strip which was prepared as  
14 in Example 1, was then brought into contact for 10  
15 minutes with the solution in the well, the hybrid was  
16 captured and visualized as in Example 3.

17

18 Results

19 Twelve samples were evaluated. The same 5 samples were  
20 found positive and the same 7 samples found negative  
21 for both methods tested.

22

23 EXAMPLE 6

24 DETECTION OF HPV IN THE CDCA SYSTEM USING COLORED LATEX  
25 BEADS AS THE COLOR GENERATING REAGENT

26 Streptavidin (Sigma) was covalently bound to 0.2  $\mu$ m  
27 styrene/vinyl carboxylic acid colored beads (Bangs  
28 Laboratories Inc., Carmel, IN, USA). The binding was  
29 accomplished by the methods of Woodward et al. as  
30 described in Example 4.

31 PCR product from a clinical sample suspected to  
32 contain HPV sequences were amplified by a second PCR  
33 amplification step using h-1 sulfonated and h-2  
34 biotinylated primers as described in Example 3. Primers  
35 were excluded from the PCR reaction mixture solution  
36 using PEG solution as described in Example 1. 3  $\mu$ l of  
37 this solution was added to a well containing 0.05% of  
38 streptavidin bound beads in 1.0% gelatin, 0.3% Tween 20

1 and 0.25 M NaCl. The contact portion 20 of a strip 22 prepared as described in Example 3 was placed in the 3 well, in contact with the solution in the well. After a 4 few minutes a blue colored signal was visible in the 5 capture zone 32 of the strip 22.

6

7

#### EXAMPLE 7

8 DETECTION OF HPV SEQUENCES IN A CAPILLARY DNA  
9 CONCENTRATION ASSAY USING DNA AS A CAPTURE REAGENT

10

#### 11 a) Selection of primers

12 Primers were selected in the E6 gene of HPV/16 and had  
13 the following sequences:

14

#### Primer 1

15

5'AAGGGCGTAACCGAAATCGGT

16

17

#### Primer 2

18

5'GTTGTTTGCAGCTCTGTGC

19

20

#### 21 b) Oligonucleotide probe capture reagent

22 The oligonucleotide probe which serves as a  
23 capture reagent was selected to be complementary to the  
24 sequence of a biotinylated strand produced by the  
25 elongation of primer 2 in a PCR reaction. The following  
26 sequence was chosen:

27 CAACAACAACAAGTTTCAGGACCCACAGGAGCGACCC

28

#### 29 c) Preparation of the Nitrocellulose backed strips

30 Mylered nitrocellulose, pore size 5 microns,  
31 (Micron Separation Inc., Westboro, MA, USA) was cut  
32 into 0.5 x 3.0 cm strips. One microliter of a solution  
33 composed of 5 ng oligonucleotide probe capture reagent  
34 in 10X SSC (SSC consisting of 0.15M NaCl and 0.015M  
35 sodium citrate, pH 7.0) was applied to middle of each  
36 nitrocellulose strip forming a spot. The strips were  
37 then dried for 15 minutes at 37°C and the  
38 oligonucleotide probes were then fixed to the

1 nitrocellulose strips by exposure of the strips to UV  
2 radiation for 5 minutes.

3

4 d) Amplification of the HPV sequence

5 PCR amplification was performed in a reaction  
6 mixture of 100  $\mu$ l aliquots containing either 1,000,  
7 100, 10, 1 or 0 pg of CasKi cell DNA in the presence of  
8 1  $\mu$ g normal human placenta DNA. Each PCR reaction mix  
9 additionally contained 100 pmole of each of the primers  
10 (P1 and P2), 0.25mM of the four deoxynucleotide  
11 triphosphates, 10  $\mu$ l 10X Taq buffer and 2.5 U of Taq  
12 DNA polymerase.

13 A first DNA denaturing step of 5 minutes at 94°C  
14 was followed by 30 cycles of 1 minute denaturing at  
15 94°C, 1.5 minute annealing at 47°C. and 1.5 minute  
16 elongation at 72°C. The amplification was ended with a  
17 seven minute elongation at 72°C.

18 e) Transport and concentration of DNA

19 The concentration and capturing of target nucleic  
20 acid sequences was achieved by the following  
21 chromatography hybridization procedure:

22 50  $\mu$ l of each PCR product obtained in step d above  
23 was diluted 1:10 in 450  $\mu$ l of hybridization solution  
24 composed of 0.6M NaCl, 20mM phosphate buffer, pH 7.5,  
25 0.02% Ficoll 400 (Sigma, St. Louis, MO, USA), 0.02%  
26 gelatin and 1% PVP. The samples were boiled for 10  
27 minutes and chilled immediately on ice. 200  $\mu$ l of each  
28 solution was then transferred to the wells 14 of the  
29 apparatus 12 shown in Figs. 1-4 and the contact portion  
30 20 of each strip 22 was brought into contact with the  
31 solution in the wells 14.

32 The apparatus 12 was placed in a humid incubator  
33 (90% relative humidity) at 37°C for 25 minutes and the  
34 solution was allowed to migrate through the  
35 nitrocellulose strips forming the bibulous carrier 24.  
36 The strips 22 were then transferred to wells 16  
37 containing 100  $\mu$ l of streptavidin alkaline phosphatase  
38 conjugate diluted 1:2,500 in PBS and 0.3% Tween 20 for

1 20 minutes. The strips 22 were then transferred to  
2 wells containing a solution including 150  $\mu$ l PBS and  
3 0.3% Tween 20. The contact portion 20 of the strip 22  
4 was brought into contact with the solution for 15  
5 minutes at 37°C. Finally the strips 22 were completely  
6 immersed in a ChemiProbe<sup>tm</sup> BCIP/NBT solution for 20  
7 minutes at 37°C to provide a substrate for a  
8 chromogenic reaction. A blue colored signal in the  
9 capture zone 32 of strip 22 indicating the presence of  
10 HPV DNA.

11 It was found that HPV sequences existing in as low  
12 as 1 pg CasKi DNA can be detected by this  
13 chromatography hybridization procedure.

14 It will be appreciated by persons skilled in the  
15 art that the present invention is not limited to what  
16 has been particularly shown and described herein above.  
17 Rather the scope of the present invention is defined  
18 only by the claims which follow:

19  
20  
21  
22  
23  
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27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38



## CLAIMS

- 1  
2  
3
- 4 1. Apparatus for transport of molecules including  
5 nucleic acid sequences in a bibulous carrier comprising  
6 a dry bibulous carrier defining a capillary transport  
7 path which supports the transport of the molecules when  
8 contacted with a solution containing the molecules.  
9
- 10 2. Apparatus according to claim 1 for concentration  
11 of target molecules in a liquid sample comprising:  
12 the dry bibulous carrier wherein the target  
13 molecules include target nucleic acid sequences and are  
14 transported within the bibulous carrier by capillary  
15 action when a portion of the dry bibulous carrier  
16 contacts the liquid sample containing the target  
17 molecules; and  
18 at least one capture reagent immobilized in  
19 at least one capture zone on the dry bibulous carrier  
20 downstream of a contact portion of the bibulous carrier  
21 wherein the at least one capture reagent is capable of  
22 capturing the target molecules.  
23
- 24 3. Apparatus for separation of target molecules,  
25 including target nucleic acid sequences, from non-  
26 target nucleotides and oligonucleotides in a liquid  
27 sample containing the target molecules and the non-  
28 target nucleotides and oligonucleotides comprising:  
29 a vessel containing a compound that binds the  
30 non-target oligonucleotides; and  
31 means for transporting the target molecules  
32 from the vessel by capillary action.  
33
- 34 4. Apparatus according to claim 2 wherein the dry  
35 bibulous carrier is a nitrocellulose membrane wherein  
36 the absorption sites have been blocked to facilitate  
37 capillary transport of the target molecules.  
38

- 1 5. Apparatus according to claim 4 wherein the dry  
2 bibulous carrier is supported by a rigid frame.  
3
- 4 6. Apparatus according to claim 2 wherein an  
5 absorbent pad is fixed to the dry bibulous carrier  
6 downstream from the at least one capture zone to  
7 facilitate capillary transport of a liquid through the  
8 dry bibulous carrier.  
9
- 10 7. Apparatus according to claim 4 wherein the  
11 absorption sites of the nitrocellulose membrane are  
12 blocked by compounds selected from a group comprising  
13 macromolecules, detergents and combinations thereof.  
14
- 15 8. Apparatus according to claim 7 wherein the  
16 macromolecules include proteins.  
17
- 18 9. Apparatus according to claim 2 wherein the at  
19 least one capture reagent comprises an antibody to a  
20 modified portion of the target nucleic acid sequence.  
21
- 22 10. Apparatus according to claim 2 wherein the at  
23 least one capture reagent comprises at least one  
24 nucleic acid capture reagent including nucleic acid  
25 probe sequences complementary to at least part of the  
26 target nucleic acid sequences.  
27
- 28 11. Apparatus according to claim 10 wherein the  
29 nucleic acid probe sequences include DNA sequences.  
30
- 31 12. Apparatus according to claim 10 wherein the  
32 nucleic acid probe sequences include RNA sequences.  
33
- 34 13. Apparatus according to claim 1 wherein the target  
35 molecules include target nucleic acid sequences  
36 comprising more than 30 base pairs.  
37
- 38 14. Apparatus according to claim 2 wherein the target

1 molecules including nucleic acid sequences comprise a  
2 nucleic acid product of an enzymatic amplification  
3 reaction and incorporate at least one pair of  
4 oligonucleotide primers.

5

6 15. Apparatus according to claim 14 wherein the at  
7 least one pair of primers comprise primers for a  
8 polymerase chain reaction (PCR).

9

10 16. Apparatus according to claim 14 wherein the at  
11 least one pair of primers comprise primers for a  
12 ligase chain reaction (LCR).

13

14 17. Apparatus according to claim 14 wherein at least a  
15 second primer of the at least one pair of primers  
16 includes an oligonucleotide bearing a ligand which  
17 binds to a at least one capture reagent whereby the  
18 target molecules which include the at least one primer  
19 bearing the ligand may be bound to the at least one  
20 capture reagent.

21

22 18. Apparatus according to claim 17 wherein the  
23 ligand comprises an antigenic epitope.

24

25 19. Apparatus according to claim 18 wherein the ligand  
26 comprises at least one sulfonated cytosine.

27

28 20. Apparatus according to claim 3 wherein the non-  
29 target oligonucleotides comprise oligonucleotide  
30 primers not incorporated in the target nucleic acid  
31 sequences.

32

33 21. Apparatus according to claim 3 wherein the  
34 compound comprises gel filtration particles too large  
35 to be transported by the means for transporting.

36

37 22. Apparatus according to claim 3 wherein the  
38 compound comprises a matrix unable to be transported by

1 the means for transporting and wherein the compound  
2 hybridizes to the non-target oligonucleotide.

3 23. A method for transport of molecules including  
4 nucleic acid sequences in a bibulous carrier comprising  
5 the steps of:

6 providing a dry bibulous carrier defining a  
7 capillary transport path which supports the transport  
8 of molecules including nucleic acid sequences; and

9 contacting the dry bibulous carrier with a  
10 solution containing molecules including nucleic acid  
11 sequences.

12

13 24. A method for concentration of molecules, including  
14 nucleic acid sequences, in a liquid sample comprising  
15 the steps of:

16 providing a dry bibulous carrier wherein the  
17 molecules are target molecules including target nucleic  
18 acid sequences and wherein the molecules are  
19 transported within the bibulous carrier by capillary  
20 action when a portion of the dry bibulous carrier  
21 contacts the liquid sample containing the molecules;

22 contacting a portion of the dry bibulous  
23 carrier with the liquid sample containing the target  
24 molecules wherein the dry bibulous carrier, when wet,  
25 defines a liquid transport path which supports the  
26 transport of molecules including nucleic acid  
27 sequences;

28 transporting the target molecules along the  
29 liquid transport path; and

30 capturing the target molecules with at least  
31 one capture reagent immobilized in at least one  
32 capture zone on the dry bibulous carrier downstream of  
33 the portion of bibulous carrier contacting the liquid  
34 sample.

35

36 25. A method for separation of target molecules,  
37 including target nucleic acid sequences, from non-  
38 target nucleotides and oligonucleotides, in a liquid

1 sample containing the target molecules and the non-  
2 target nucleotides and oligonucleotides comprising the  
3 steps of:

4 providing a vessel containing a compound that  
5 binds the non-target oligonucleotides;

6 adding the liquid sample which includes the  
7 target molecules and the non-target nucleotides and  
8 oligonucleotides; and

9 transporting the target molecules by  
10 capillary action.

11

12 26. Apparatus for separation of target molecules,  
13 including target nucleic acid sequences, from non-  
14 target nucleotides and oligonucleotides in a liquid  
15 sample containing the target molecules and the non-  
16 target nucleotides and oligonucleotides, concentration  
17 of the target molecules, and detection of the  
18 concentrated target molecules comprising:

19 a vessel apparatus defining a plurality of  
20 wells including a first portion of the plurality of  
21 wells containing a compound that binds the non-target  
22 oligonucleotides and wherein the liquid sample may be  
23 added to the first portion of the plurality of wells;

24 a dry bibulous carrier defining a liquid  
25 transport path from the vessel that when wet supports  
26 the transport of the target molecules wherein the  
27 target molecules are transported within the bibulous  
28 carrier by capillary action when a contact portion of  
29 the dry bibulous carrier contacts the liquid sample  
30 containing the target molecules;

31 at least one capture reagent capable of  
32 capturing the target molecules wherein the at least  
33 one capture reagent is immobilized in at least one  
34 capture zone on the dry bibulous carrier downstream of  
35 the contact portion of the bibulous carrier; and

36 means for detecting the captured target  
37 molecules.

38

1 27. A method for concentration and detection of  
2 target nucleic acid sequences, in a liquid sample  
3 comprising the steps of:

4           providing a dry bibulous carrier wherein the  
5 target nucleic acid sequences are transported within  
6 the bibulous carrier by capillary action when a portion  
7 of the dry bibulous carrier contacts the liquid sample  
8 containing the target nucleic acid sequences;

9           contacting a portion of the dry bibulous  
10 carrier with the liquid sample containing the target  
11 nucleic acid sequences wherein the dry bibulous  
12 carrier, when wet, defines a liquid transport path  
13 which supports the transport of the target nucleic acid  
14 sequences;

15           transporting the target nucleic acid  
16 sequences along the liquid transport path; and

17           capturing the target nucleic acid sequences  
18 by hybridization with at least one nucleic acid capture  
19 reagent immobilized in at least one capture zone on the  
20 dry bibulous carrier downstream of the portion of  
21 bibulous carrier contacting the liquid sample.

22

23 28. Apparatus for concentration and detection of  
24 target nucleic acid sequences comprising:

25       a vessel apparatus defining a plurality of wells;

26       a dry bibulous carrier defining a liquid  
27 transport path from the vessel that when wet supports  
28 the transport of the target nucleic acid sequences  
29 wherein the target nucleic acid sequences are  
30 transported within the bibulous carrier by capillary  
31 action when a contact portion of the dry bibulous  
32 carrier contacts the liquid sample containing the  
33 target nucleic acid sequences;

34       at least one nucleic acid capture reagent  
35 including nucleic acid probe sequences for capturing  
36 the target nucleic acid sequences by hybridization and  
37 wherein the at least one nucleic acid capture reagent  
38 is immobilized in a capture zone on the dry bibulous

1 carrier downstream of the contact portion of the  
2 bibulous carrier; and  
3 means for detecting the captured the target  
4 nucleic acid sequences.

5

6 29. Apparatus according to claim 26 wherein the means  
7 for detecting comprises:

8 a bibulous carrier upon which target  
9 molecules, including nucleic acid sequences, bearing a  
10 ligand which binds to a signal producing reagent are  
11 immobilized; and

12 means for contacting the target molecules,  
13 including the nucleic acid sequences, bearing the  
14 ligand with the signal producing reagent to produce a  
15 sensible signal indicating the detection of the target  
16 molecules including the nucleic acid sequences.

17

18 30. Apparatus according to claim 29 wherein the target  
19 nucleic acid sequences are the product of an enzymatic  
20 amplification reaction and incorporate at least one  
21 pair of oligonucleotide primers.

22

23 31. Apparatus according to claim 26 wherein the non-  
24 target oligonucleotides comprise oligonucleotide  
25 primers not incorporated in the target nucleic acid  
26 sequences.

27

28 32. Apparatus according to claim 30 wherein the at  
29 least one pair of primers comprise primers for a  
30 polymerase chain reaction  
31 (PCR).

32

33 33. Apparatus according to claim 30 wherein the one  
34 pair of primers comprise primers for a ligase chain  
35 reaction (LCR).

36

37 34. Apparatus according to claim 30 where a second  
38 primer of the at least one pair of oligonucleotide

1 primers includes a ligand which binds to the at least  
2 one capture reagent whereby the target molecules that  
3 include the ligand may be bound to the at least one  
4 capture reagent.

5

.6 35. Apparatus according to claim 34 wherein the ligand  
7 comprises an antigenic epitope.

8

9 36. Apparatus according to claim 35 wherein the ligand  
10 comprises at least one sulfonated cytosine.

11

12 37. Apparatus according to claim 30 where a first  
13 primer of the at least one pair of primers includes a  
14 ligand which binds to a signal producing reagent  
15 whereby the target molecules that include the ligand  
16 may be detected by the presence of a signal produced by  
17 the signal producing reagent.

18

19 38. Apparatus according to claim 37 where a first  
20 primer of the at least one pair of primers includes a  
21 ligand which binds to a signal producing reagent  
22 whereby the target molecules that include the ligand  
23 may be detected by the presence of a signal produced by  
24 the signal producing reagent after contacting a signal  
25 developing reagent.

26

27 39. Apparatus according to claim 37 wherein the ligand  
28 comprises biotinylated nucleotides.

29

30 40. Apparatus according to claim 37 wherein the signal  
31 producing reagent comprises streptavidin linked to  
32 colored latex beads.

33

34 41. Apparatus according to claim 38 wherein the signal  
35 produced by the signal producing reagent after  
36 contacting the signal developing reagent includes a  
37 streptavidin-alkaline phosphatase conjugate.

38



1 42. Apparatus according to claim 26 wherein the first  
2 portion of wells also contains the signal producing  
3 reagent.

4

5 43. Apparatus according to claim 26 wherein the  
6 plurality of wells additionally includes a second  
7 portion of the wells containing a washing solution.

8

9 44. Apparatus according to claim 26 wherein the  
10 plurality of wells also includes a third portion of the  
11 wells containing a signal developing reagent solution.

12

13 45. Apparatus according to claim 28 wherein the  
14 plurality of wells comprise a first portion of wells  
15 containing a sample to be tested for the target nucleic  
16 acid sequences.

17

18 46. Apparatus according to claim 28 wherein the  
19 plurality of wells additionally comprises a second  
20 portion of the wells containing the signal producing  
21 reagent.

22

23 47. Apparatus according to claim 28 wherein the  
24 plurality of wells additionally comprises a third  
25 portion of wells containing a washing solution.

26

27 48. Apparatus according to claim 28 wherein the  
28 plurality of wells additionally comprises a fourth  
29 portion of wells containing a signal developing  
30 reagent.

31

32 49. Apparatus according to claim 26 wherein the dry  
33 bibulous carrier comprises at least one strip.

34

35 50. Apparatus according to claim 49 wherein each of  
36 the first portion of wells are adapted to receive the  
37 contact portion of each strip to permit transport of  
38 the target molecules to the at least one capture zone

1 where they are captured.

2

3 51. Apparatus according to claim 43 wherein each of  
4 the second portion of wells is adapted to receive the  
5 contact portion of each strip for washing the strip to  
6 remove no specifically captured compounds after  
7 immobilization of the target molecules in the at least  
8 one capture zone.

9

10 52. Apparatus according to claim 44 wherein each of  
11 the third portion of wells is adapted to receive an  
12 entire strip.

13

14 53. Apparatus according to claim 52 wherein the means  
15 for contacting comprises:

16 at least one of the third portion of wells  
17 containing a signal producing reagent solution; and

18 at least one strip after immobilization of  
19 the target molecules in the at least one capture zone  
20 wherein the entire strip is in contact with a signal  
21 developing reagent solution permitting contact of the  
22 signal developing reagent with the at least one capture  
23 zone.

24

25 54. Apparatus according to claim 28 wherein each of  
26 the first portion of wells is adapted to receive the  
27 contact portion of each strip to permit transport of  
28 the target nucleic acid sequences to the at least one  
29 capture zone where they are captured.

30

31 55. Apparatus according to claim 46 wherein each of the  
32 second portion of wells is adapted to receive the  
33 contact portion of each strip to permit transport of  
34 the signal producing reagent to the at least one  
35 capture zone where the signal producing reagent is  
36 bound to the ligand borne on the target nucleic acid  
37 sequences.

38

1 56. Apparatus according to claim 47 wherein each of  
2 the third portion of wells is adapted to receive the  
3 contact portion of each strip for washing the strip to  
4 remove non-specifically captured compounds after  
5 immobilization of the target nucleic acid sequences in  
6 the at least one capture zone.

7

8 57. Apparatus according to claim 48 wherein the means  
9 for contacting comprises:

10 at least one of the fourth portion of wells  
11 containing a signal developing reagent; and

12 at least one strip after immobilization of  
13 the target nucleic acid sequences in the at least one  
14 capture zone wherein the entire strip is in contact  
15 with the signal developing reagent solution permitting  
16 contact of the signal developing reagent with the at  
17 least one capture zone.

18

19 58. Apparatus according to claim 57 wherein each of  
20 the fourth portion of wells is adapted to receive an  
21 entire strip.

22

23 59. A method for the detection of a specific nucleic  
24 acid sequence comprising the steps of:

25 amplifying by an enzymatic reaction at least  
26 a portion of an original nucleic acid sequence to  
27 produce target molecules including nucleic acid  
28 sequences which are specific to the at least a portion  
29 of the original nucleic acid sequence;

30 separating the target molecules from non-  
31 target nucleotides and oligonucleotides including the  
32 steps of:

33 providing a vessel containing a substrate  
34 that binds the non-target nucleotides and  
35 oligonucleotides;

36 adding a liquid sample which includes the  
37 target molecules and the non-target nucleotides and  
38 oligonucleotide;

1                   and transporting the target molecules by  
2 capillary action;  
3                   concentrating the target molecules including  
4 the steps of:  
5                   providing a dry bibulous carrier wherein the  
6 target molecules are transported within the bibulous  
7 carrier by capillary action when a portion of the dry  
8 bibulous carrier contacts the liquid sample containing  
9 the target molecules;  
10                  contacting a portion of the dry bibulous  
11 carrier with the liquid sample containing the target  
12 nucleic acid sequences wherein the dry bibulous  
13 carrier, when wet, defines a liquid transport path  
14 which supports the transport of the target molecules;  
15                  transporting the target molecules along the  
16 liquid transport path; and  
17                  capturing the target molecules with at  
18 least one capture reagent immobilized in a capture  
19 zone on the dry bibulous carrier downstream of the  
20 portion of bibulous carrier contacting the liquid  
21 sample; and  
22                  detecting the target molecules by contacting  
23 target molecules having a ligand which binds to a  
24 signal producing reagent and are immobilized on a  
25 bibulous carrier with a signal developing reagent to  
26 produce a sensible signal.  
27  
28 60. A method for the detection of a specific nucleic  
29 acid sequence comprising the steps of:  
30                  amplifying by an enzymatic reaction at least  
31 a portion of an original nucleic acid sequence to  
32 produce target nucleic acid sequences which are  
33 specific to the at least a portion of the original  
34 nucleic acid sequence;  
35                  providing a liquid sample which includes the  
36 target nucleic acid sequences;  
37                  transporting the target nucleic acid  
38 sequences by capillary action;

1           concentrating the target nucleic acid  
2 sequences including the steps of:

3           providing a dry bibulous carrier wherein the  
4 target nucleic acid sequences are transported within  
5 the bibulous carrier by capillary action when a portion  
6 of the dry bibulous carrier contacts the liquid sample  
7 containing the target nucleic acid sequences;

8           contacting a portion of the dry bibulous  
9 carrier with the liquid sample containing the target  
10 nucleic acid sequences wherein the dry bibulous  
11 carrier, when wet, defines a liquid transport path  
12 which supports the transport of the target nucleic acid  
13 sequences; and

14          transporting the target nucleic acid  
15 sequences along the liquid transport path;

16          capturing the target nucleic acid sequences  
17 with at least one nucleic acid capture reagent  
18 immobilized in at least one capture zone on the dry  
19 bibulous carrier downstream of the portion of bibulous  
20 carrier contacting the liquid sample; and

21          detecting the target nucleic acid sequences  
22 by contacting target nucleic acid sequences having a  
23 ligand which binds to a signal producing reagent and  
24 are immobilized on a bibulous carrier with a signal  
25 developing reagent to produce a sensible signal.

26

27

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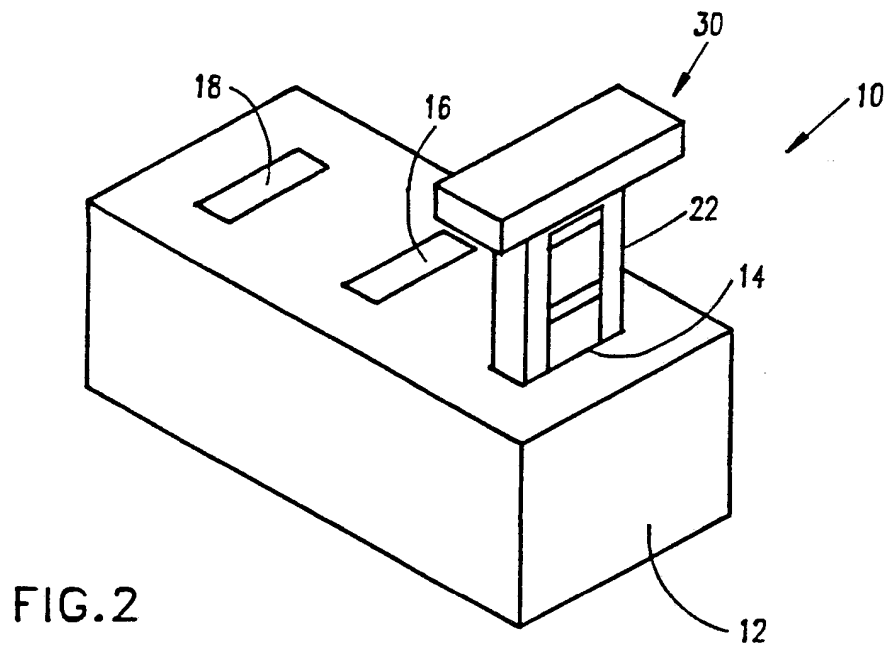
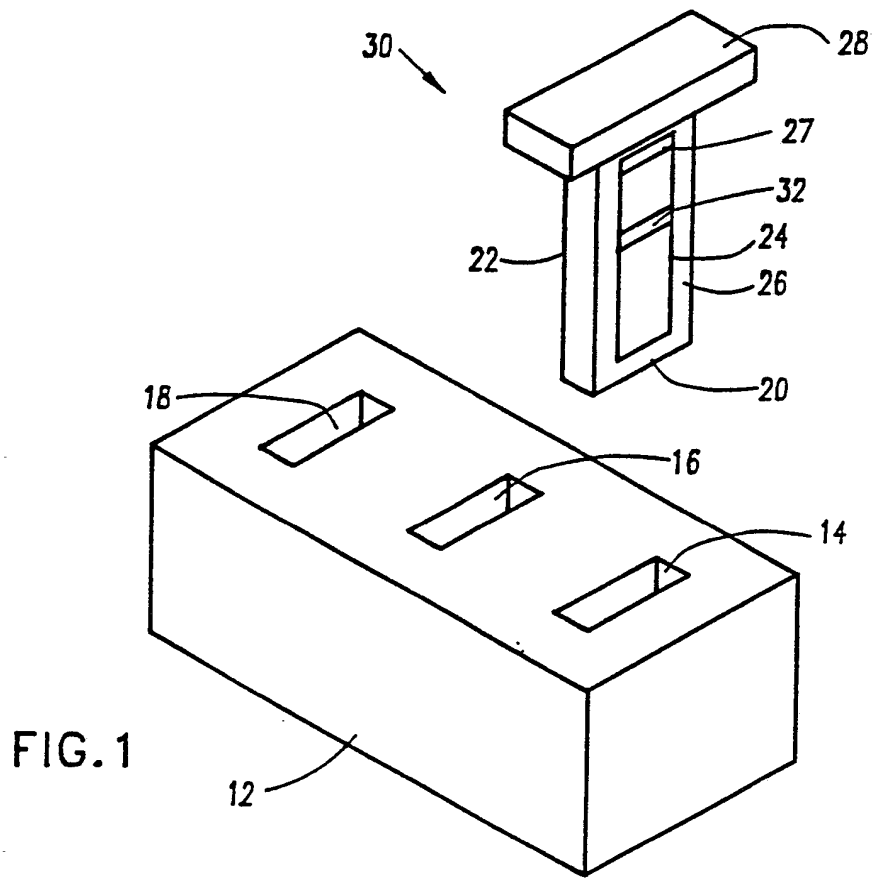
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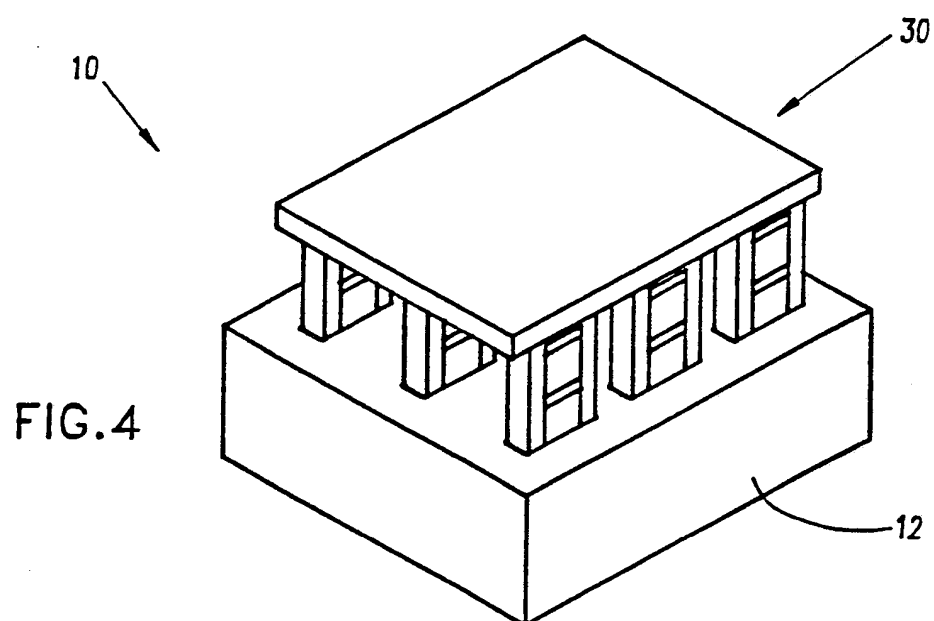
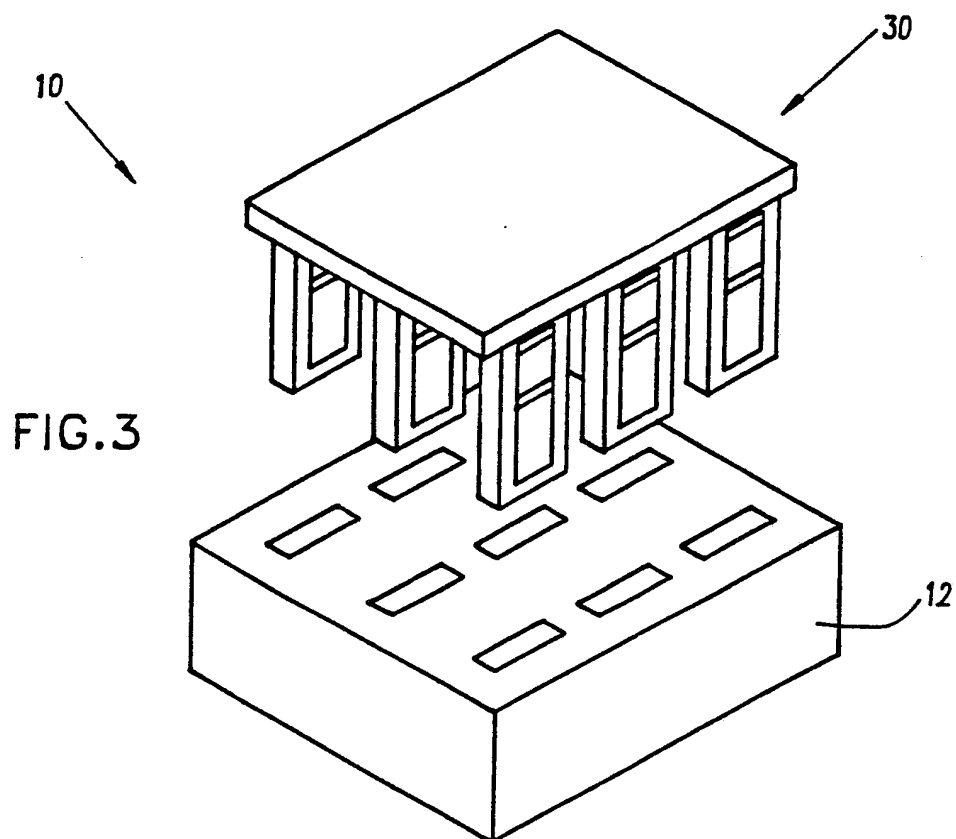
36

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 92/00176

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all)<sup>6</sup>

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12Q1/68; G01N33/558; G01N33/543; // C12Q1/70

**II. FIELDS SEARCHED**Minimum Documentation Searched<sup>7</sup>

Classification System

Classification Symbols

Int.Cl. 5

C12Q ; G01N

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched<sup>8</sup>**III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup>**

Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	EP,A,0 262 328 (ABBOTT LABORATORIES) 6 April 1988  see page 9, line 1 - page 15, line 52 see page 28, line 35 - line 47 see page 32, line 10 - page 33, line 52 see page 41, line 20 - page 42, line 50; claims  ---	1-5, 23-25, 27, 49, 50
X	EP,A,0 306 336 (SYNTEX (USA) INC.) 8 March 1989 see page 5, line 2 - line 19 see page 7, line 56 - page 8, line 18 see page 11, line 50 - page 12, line 39 see page 19, line 60 - page 21, line 61; claims  ---  -/--	1

<sup>10</sup> Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

**IV. CERTIFICATION**

Date of the Actual Completion of the International Search

15 JANUARY 1993

Date of Mailing of this International Search Report

28. 01. 93

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

LUZZATTO E.R.



III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
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A	WO,A,8 910 979 (E.I. DU PONT DE NEMOURS AND COMPANY) 16 November 1989 see page 7, line 15 - page 16, line 16; claims ---	2,14,17
A	EP,A,0 318 255 (EASTMAN KODAK COMPANY) 31 May 1989 see the whole document ---	29-32,60
A	WO,A,9 106 659 (PBS-ORGENICS) 16 May 1991 see the whole document ---	18,19, 26,36
A	EP,A,0 362 809 (BOEHRINGER BIOCHEMIA ROBIN S.P.A.) 11 April 1990 see the whole document ---	1-4
A	GB,A,2 191 577 (LANCE ALLEN LIOTTA) 16 December 1987 see the whole document -----	3,21,22

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

NL 9200176  
SA 65378

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

15/01/93

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